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ANOMALOUS SEDIMENTATION BEHAVIOUR OF THE E.COLI RIBOSOMAL SYSTEM: 50S–30S ⇄ 50S + 30S

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An experimental and theoretical study has been made into the effect of association–dissociation reactions on the sedimentation of the E.coli ribosomal system 50S–30S ⇌ 50S+30S. It has been found that

(a) the sedimentation pattern is strongly dependent on the rotor speed;

(b) the ratio of components as measured using high-speed ultracentrifugation (30000–40000 r.p.m.) is independent of rotor speed; and

(c) the speed of ultracentrifugation has a strong effect on the sedimentation coefficient of the ribosomal system as determined by the mean square second moment.

The results of this paper demonstrate that ribosome sedimentation at low-speed ultracentrifugation is affected by some artefactual processes. A theoretical analysis of the experimental findings has shown that the observed effects cannot be attributed to the effect of the association–dissociation reaction nor to the pressure dependence of the equilibrium constant of that reaction.

On the other hand, at high-speed ultracentrifugation the ribosomal system sediments as a heterogeneous mixture of non-interacting components. Consequently, the shape of the boundary in this case will reflect the equilibrium composition of the ribosomal system.

1. Introduction

The reaction mixture of "purified" 50S and 30S ribosomal subparticles of E. coli has been shown to behave as a reversibly interacting system under certain conditions [1,2]. Sedimentation velocity studies of such a system are complicated by the fact that the association–dissociation reactions may materially affect the separation of components. In particular, the boundary observed in transport experiments with a reversibly interacting system do not necessarily reflect the actual number of components present [1,3]. Moreover, the boundary shape depends on the relation between the rates of species separation and chemical reactions [4–6]. When the rate of component separation becomes much greater than the reaction rates, the system sediments in a manner analogous to a heterogeneous mixture of non-interacting components whose apparent concentration ratio corresponds to the equilibrium established in the system prior to the analysis.

The purpose of the present investigation was to explore the effect of association–dissociation reactions on the sedimentation properties of the E.coli ribosomal system 50S–30S⇌50S+30S at different speeds of the rotor. It has been found that the sedimentation coefficient, as measured by the mean square second moment, and the sedimentation patterns of the E. coli ribosomal system both strongly depend on the rotor speed. The ratio of the components, measured using high-speed ultracentrifugation (30000–40000 r.p.m.) is independent of rotor speed.

However, a theoretical analysis of the experimental results has indicated that the observed effects cannot be attributed to the association–dissociation reaction,

nor to the pressure dependence of the equilibrium constant of that reaction. Possible reasons for the observed dependence of the ribosome sedimentation on the applied force are discussed.

2. Materials and methods

2.1. Preparation of ribosomes

The preparation of E. coli MRE 600 ribosomal subparticles dissolved in a standard buffer – 1 mM K_2HPO_4, 50 mM KCl – containing 20 mM Mg^{2+} (pH 7.5) has been described previously [2]. For reassociation, the subparticles were mixed in equimolar proportions, the final ribosome concentration was 22 A_{260} unit/ml. This reaction mixture was then divided into two parts and diluted with a standard buffer to a final ribosome concentration of 1 A_{260} unit/ml and a Mg^{2+} concentration of 5 mM and 20 mM respectively. After incubation for 30 min at 20°C a few samples of reassociated ribosomes dissolved in 20 mM Mg^{2+} were fixed by addition of formaldehyde to a final concentration of 4%. The samples of 50S subparticles (1 A_{260} unit/ml) dissolved in a standard buffer containing 5 mM Mg^{2+} were prepared by an appropriate dilution of the initial 50S subparticles suspension. The temperature was kept at 4°C throughout these treatments. Sedimentation analysis was performed after incubating the solutions for 1 hour at 20°C.

2.2. Analytical ultracentrifugation

Sedimentation velocity studies of the ribosomal preparations were carried out in a Spinco Model E (Beckman) analytical ultracentrifuge equipped with a split-beam photoelectric scanner, a monochromator and a multiplexer, enabling simultaneous analysis of several samples to be made in a multichannel rotor. Sedimenting boundaries were monitored at the wavelength of 260 nm. Double-sector cells (12 mm) were used, which were washed after each run, with a buffer corresponding to the test preparation and filled with fresh portions of ribosomal solution. Cells were positioned in the rotor by the use of a microscope cell aligner. Several portions of each sample were centrifuged at different rotor speeds in the range from 12000 to 40000 r.p.m. at 20°C.

The position of a moving boundary was determined by the expression corresponding to the square-root of the second moment of the concentration gradient curve [7,8]:

$$r = \sqrt{\int_{C_m}^{C_p} r^2 \mathrm{d}C \Big/ \int_{C_m}^{C_p} \mathrm{d}C},$$

where r is the distance from the axis of rotation, C_m, C_p are the ribosome concentrations (weight scale) at the meniscus and at a certain point in the plateau region, and C is the ribosome concentration (weight scale).

Sedimentation coefficients were calculated from the slopes of the linear plots (fitted by the method of least squares) of the logarithm of boundary position versus time. The correlation coefficient exceeded 0.9995 in most cases.

3. Results and discussion

3.1. Effect of rotor speed on the sedimentation pattern of E. coli ribosomal preparations

Fig. 1 shows the typical sedimentation patterns obtained by analytical centrifugation of the E. coli ribosomal preparations (see legend for fig. 1) at different rotor speeds. As seen in fig. 1, at the speed of 12000 r.p.m., the preparations sediment with a single, approximately symmetrical and not very diffuse boundary. At 16000 r.p.m. the boundary becomes heterogeneous but the components distinctly separate only at 22000 r.p.m. or higher.

This effect has been reported for E. coli ribosomes earlier [1] and has been interpreted qualitatively as resulting from the influence of the association–dissociation reaction of ribosomes on the separation of components. In fact, as shown in appendix 1, the association–dissociation reactions can distort the separation of the components. This effect depends on the rotor speed and vanishes at sufficiently high speed of ultracentrifugation when the following inequalities are satisfied:

$$k_d T \ll 1, \quad k_a C_1^0 T \ll 1, \quad k_a C_2^0 T \ll 1,$$

where k_a, k_d are the rate constants for the association and dissociation of the subparticles, C_1^0, C_2^0 are the

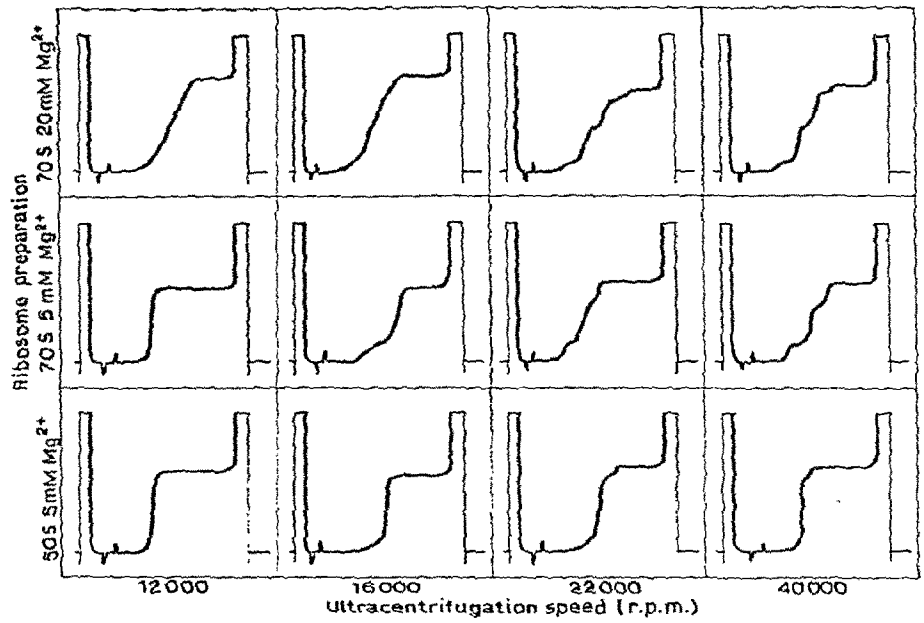

Fig. 1. Sedimentation patterns obtained at analytical ultra-centrifugation of different ribosomal preparations at indicated rotor speeds (20°C).

70S 20 mM Mg^{2+} – equimolar mixture of purified 30S and 50S subparticles in a buffer containing 0.001 M K_2HPO_4, 50 mM KCl and 20 mM Mg^{2+} (pH 7.5).

70S 5 mM Mg^{2+} – equimolar mixture of purified 30S and 50S subparticles in a buffer containing 1 mM K_2HPO_4, 50 mM KCl and 5 mM Mg^{2+} (pH 7.5).

50S 5 mM Mg^{2+} – solution of 50S subparticles in a buffer containing 1 mM K_2HPO_4, 50 mM KCl and 5 mM Mg^{2+} (pH 7.5).

equilibrium concentrations of 30S and 50S subparticles capable of associating, T is the optimal time of ultracentrifugation.

At the rotor speeds above 22 000 r.p.m., the components are distinctly separated (see fig. 1), and their concentrations can be measured directly on the sedimentation patterns. The concentration ratios of components determined taking into account the radial dilution are presented in table 1, from which it may be seen that the ratios are independent of the rotor

Table 1
Concentration ratio of components as determined by high-speed ultracentrifugation of different ribosomal preparations

Ultra-centrifugation speed (r.p.m.)	Relative amount of components in %							
	in mixture of "purified" 30S and 50S subparticles at 20 mM Mg^{2+}			in mixture of "purified" 30S and 50S subparticles at 5 mM Mg^{2+}			in solution of 50S subparticles at 5 mM Mg^{2+}	
	30S	50S	70S–100S	20S	50S	70S	50S	dimers (70S)
26000	–	–	–	–	–	–	88	12
30000	7	40	53	18	53	29	–	–
36000	8	39	53	20	50	30	87	13
40000	8	38	54	18	51	31	87	13

speed at high-speed ultracentrifugation. It appears that the effect of the ribosomal association–dissociation reaction is so small in this case that the ribosomal system sediments as a heterogeneous mixture of non-interacting components. This being so, the concentration ratio of components determined by high-speed ultracentrifugation will correspond to the chemical equilibrium that had been established in the system before the beginning of sedimentation, i.e., it will be rotor speed independent. It should be noted that in the process of analytical ultracentrifugation the true ratio of components in the plateau region remains practically constant and corresponds within the limits of experimental error to the state of the system prior to analysis, regardless of the rotor speed and the rates of chemical reactions (see section 3.2).

3.2. Rotor speed dependence of the sedimentation coefficient

The sedimentation coefficient of E. coli ribosomal preparations was determined by a method analogous to the measurement of the mean square second moment of the concentration gradient curve [7,8]. For each sample and for each rotor speed, several ultracentrifugation runs were made (see section 2), and the mean value of sedimentation coefficient was calculated. The mean square error did not exceed 1 S in most cases.

Figs. 2, 3 and 4 show the sedimentation coefficients plotted against rotor speeds for three ribosomal preparations (see legends to the figures). It will be seen that rotor speed has a strong effect on the sedimentation coefficient, the character of this effect being the same for all the preparations tested. However, the variations of the sedimentation coefficient is greater in the case of ribosomal preparations in a buffer with a low (5 mM) Mg^{2+} concentration.

The speed of ultracentrifugation has been previously reported to affect the sedimentation coefficient of certain macromolecules [9–14]. This phenomenon is rather difficult to interpret because the sedimentation coefficient depends on a multitude of interrelated variables whose effects might in turn be determined by the rotor speed. Also, the effect is not universal and depends to a large extent on the particular properties of the systems used. Attempts to explain this phenomenon in terms of molecular hydrodynamic effects (orientation of asymmetrical macromolecules [9], the

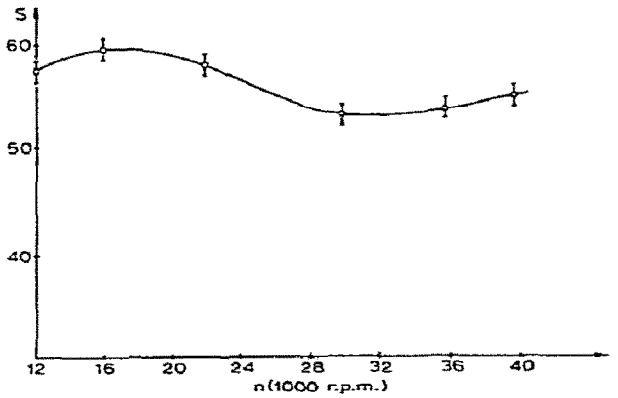

Fig. 2. Effect of rotor speed on the sedimentation coefficient of an equimolar mixture of purified 30S and 50S subparticles in a buffer containing 1 mM K_2HPO_4, 50 mM KCl and 20 mM Mg^{2+} (pH 7.5).

deformation and subsequent orientation [15], the influence of hydrostatic pressure on the density and viscosity of the solvent and the partial specific volume of macromolecule [9–12], solvent counterflow, etc.) have failed the expected effects being rather insignificant.

Let us consider now the effect of the association–dissociation reaction on ribosome sedimentation. Earlier, on the basis of general considerations, it has been suggested, that the sedimentation coefficient of

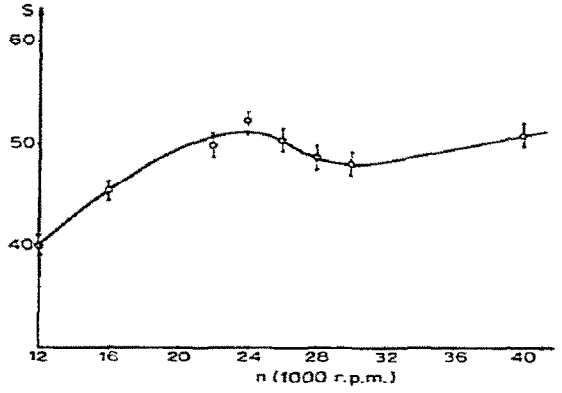

Fig. 3. Effect of rotor speed on the sedimentation coefficient of an equimolar mixture of purified 30S and 50S subparticles in a buffer containing 1 mM K_2HPO_4, 50 mM KCl and 5 mM Mg^{2+} (pH 7.5).

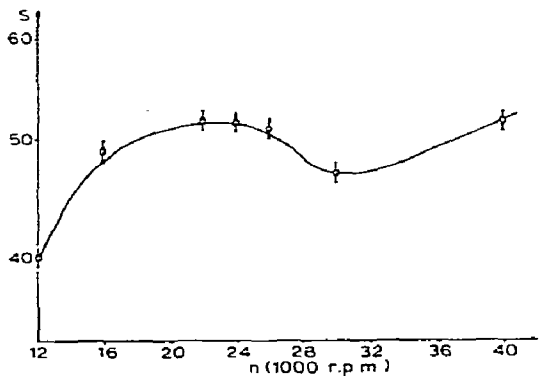

Fig. 4. Effect of rotor speed on the sedimentation coefficient of 50S subparticles in a buffer containing 1 mM K_2HPO_4, 50 mM KCl and 5 mM Mg^{2+} (pH 7.5).

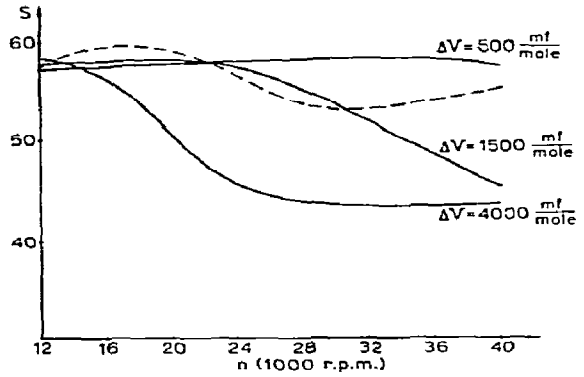

Fig. 5. Effect of rotor speed on the sedimentation coefficient of the reversibly interacting system AB $\rightleftarrows$ A + B whose equilibrium constant is pressure dependent. Parameters used for the calculations are given in appendix 3.

self-associating systems is rotor speed independent [9–13]. Using the transport equations for the reversibly interacting ribosomal system, it can be shown (see appendix 2) that the sedimentation coefficient of such a system as measured by the mean square second moment, is equal, with good approximation, to the weight average sedimentation coefficient corresponding to the distribution of components established in the system before sedimentation and does not depend on the rotor speed provided that $\omega^2 T = \text{const}$. It is to be noted that the result is a consequence of the fact that the ratio of components in the plateau remains practically unchanged in the process of analytical ultracentrifugation of reversibly interacting systems, regardless of the rotor speed.

For E. coli ribosomes, a pressure dependence of the equilibrium constant of the association–dissociation reaction has been suggested [16]. In this case the sedimentation coefficient of the system may, generally speaking, be rotor speed dependent [10, 17]. Fig. 5 shows the results of a theoretical consideration of the effect of ultracentrifugation speed on the sedimentation coefficient of the equilibrium reversibly interacting system AB $\rightleftarrows$ A + B with a pressure dependent equilibrium constant (see appendix 3). It can be seen that in a certain region of low rotor speeds, the sedimentation coefficient remains practically constant (in contrast to the experimental data of figs. 2, 3 and 4) and is approximately equal to the weight average sedimentation coefficient of the system corresponding to the component ratio observed at atmospheric pressure.

Further increase of the rotor angular velocity results in a decrease of the sedimentation coefficient. The least (asymptotic) value of this coefficient is equal to the weight average sedimentation coefficient of the system in the event of complete dissociation. It should be noted, however, that in high-speed ultracentrifugation, the ribosomal system does not appear to be in equilibrium any more, and so it sediments as a heterogeneous mixture of non-interacting components (the separation of components is apparent (see fig. 1), and the concentration ratio is rotor speed independent); also, no complete dissociation is observed (see fig. 1). For comparison, fig. 5 shows the experimental curve of the sedimentation coefficient versus rotor speed (dotted line) for one of the ribosomal preparations (see fig. 2). It appears that the decrease of the sedimentation coefficient in the speed region of 20000 to 30000 r.p.m. may be partly due to the pressure dependence of the equilibrium constant. It is obvious, however, that the expected and observed effects are highly discrepant quantitatively.

Therefore, the experimental data of figs. 2, 3 and 4 cannot be accounted for by association–dissociation reaction. Moreover, the sedimentation properties of formaldehyde-treated ribosomal preparations subjected to low- and high-speed ultracentrifugation, have likewise been shown to be strongly dependent on the rotor speed: the sedimentation coefficient of such a heterogeneous non-reactive system is 29S at 12000 r.p.m. and 45S at 40000 r.p.m. As known, formaldehyde-

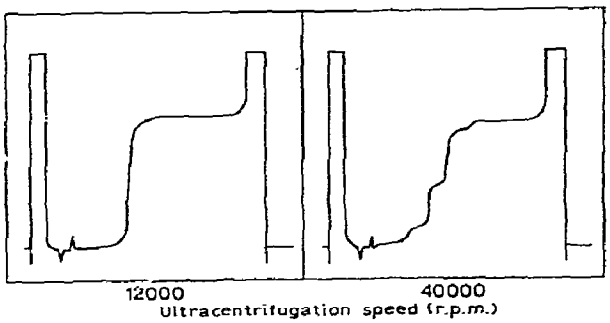

Fig. 6. Sedimentation patterns obtained at low-speed and high-speed ultracentrifugation (20°C) of a formaldehyde-treated equimolar mixture of purified 30S and 50S subparticles in a buffer containing 1 mM K_2HPO_4, 50 mM KCl and 20 mM Mg^{2+} (pH 7.5).

treated subparticles are no longer able to associate, and 50S–30S associate is incapable of dissociating after formaldehyde treatment [18–20], i.e., the ribosomal system becomes a heterogeneous mixture of "non-interacting" components. It is to be noted also that formaldehyde fixation of the ribosomal system is a rather fast process in comparison with association–dissociation reactions [21]. Fig. 6 illustrates sedimentation profiles obtained by ultracentrifugation of a formaldehyde-fixed equilibrium ribosomal system. As can be seen from fig. 6, at low-speed ultracentrifugation (12000 r.p.m.) the fixed ribosomal system sediments with a single and rather sharp boundary, while at the speed of 40000 r.p.m. the components are distinctly separated. This effect seems surprising, since separation of the components of the ideal (independent sedimentation of the components, absence of chemical reaction) heterogeneous system does not depend on the speed of ultracentrifugation provided that $\omega^2 T = \text{const}$. (see, for example, appendix 1). Although the results of fig. 6 are difficult to explain at present, they show that the rotor speed dependence of sedimentation patterns may be due not only to ribosomal association–dissociation reactions but also to some other processes, the nature of which is as yet unknown.

Analysis has shown that none of the considered sedimentation models of the ribosomal system is consistent with the effects observed. Among other factors responsible for the anomalous ribosome sedimentation at low-speed ultracentrifugation the most probable seems to be convective flows induced by small temperature gradients [22], or the rotor speed dependent immobilization of the solvent by the suspended particles [23]. However, further elucidation of the nature of this complicated phenomenon is beyond the scope of the present investigation, which purpose was to explore the effect of association–dissociation reactions on the ribosome sedimentation. Finally, it is to be noted that experimental and theoretical data reported in this communication show that: (i) anomalous ribosome sedimentation at low-speed ultracentrifugation is not a consequence of association–dissociation reactions only [1], but is largely conditioned by some molecular-hydrodynamic factors, (ii) at high-speed ultracentrifugation (30000–40000 r.p.m.) the effect of association–dissociation reactions and of molecular-hydrodynamic factors, which play a substantial role at low-speed centrifugation is practically negligible [the shape of the boundary does not depend on the rotor speed provided that $\omega^2 T = \text{const}$. (table 1, fig. 1)], though the sedimentation coefficient of the ribosomal system in this region of the rotor varies slightly (figs. 2, 3 and 4).

These results allow us to suggest that components concentrations determined by high-speed ultracentrifugation will correspond to the equilibrium established in the ribosomal system before analysis.

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Appendix 1

Effect of the association–dissociation reaction on the separation of components at analytical ultracentrifugation of the reversibly interacting system $AB \rightleftarrows A + B$

Let us introduce a parameter characterizing the separation of components

$$Z_{ik} = \ln \tilde{r}_i / \tilde{r}_k \,, \tag{1}$$

where Z_{ik} is the parameter characterizing the separation of the ith and kth components, and $\tilde{r}_i$, $\tilde{r}_k$ are the distances from the axis of rotation to the point at the boundary as determined by the mean square second moments for the ith and kth components, respectively. It is assumed that $\tilde{r}_i > \tilde{r}_k$.

Evidently, Z_{ik} depends on angular velocity, ω, and ultracentrifugation time, T. We shall consider this parameter on condition that $\omega^2 T = \text{const}$. For further calculations, it is convenient to introduce another expression of Z_{ik}, identical to the expression (1):

$$Z_{ik} = \omega^2 \int_0^T (\tilde{S}_i - \tilde{S}_k)\, \mathrm{d}t \,, \tag{2}$$

where $\tilde{S}_i$ and $\tilde{S}_k$ are the sedimentation coefficients of the ith and kth components as measured by the mean square second moments.

For a heterogeneous system whose components sediments independently, it is evident that $\tilde{S}_j = S_j$, i.e., that the sedimentation coefficient of the jth component as measured by the mean square second moment, corresponds to the sedimentation coefficient of individual particles [7,8]; then

$$Z_{ik} = \omega^2 \int_0^T (\tilde{S}_i - \tilde{S}_k)\, \mathrm{d}t = \omega^2 T (S_i - S_k) \,. \tag{3}$$

Thus, as could be expected, the separation of components of a heterogeneous mixture is independent of the ultracentrifugation speed provided that $\omega^2 T = \text{const}$.

Consider now the effect of the association–dissociation reaction on the separation of components of the reversibly interacting $AB \rightleftarrows A + B$. Let us assume, for the sake of certainty the following molecular weight ratio of components: $M_A : M_B : M_{AB} = 1:2:3$. The differential equations of the sedimentation process will then take the following form:

$$\begin{aligned}
\frac{\partial C_1}{\partial t} &= D_1 \frac{\partial^2 C_1}{\partial r^2} + D_1 \frac{1}{r}\frac{\partial C_1}{\partial r} - S_1 \omega^2 r \frac{\partial C_1}{\partial r} - 2 S_1 \omega^2 C_1 + Q \,, \\
\frac{\partial C_2}{\partial t} &= D_2 \frac{\partial^2 C_2}{\partial r^2} + D_2 \frac{1}{r}\frac{\partial C_2}{\partial r} - S_2 \omega^2 r \frac{\partial C_2}{\partial r} - 2 S_2 \omega^2 C_2 + 2Q \,, \\
\frac{\partial C_3}{\partial t} &= D_3 \frac{\partial^2 C_2}{\partial r^2} + D_3 \frac{1}{r}\frac{\partial C_3}{\partial r} - S_3 \omega^2 r \frac{\partial C_3}{\partial r} - 2 S_3 \omega^2 C_3 - 3Q \,,
\end{aligned} \tag{4}$$

where C_1, C_2 and C_3 are the concentrations of components A, B and AB, respectively (weight scale); Q is the rate of concentration change of component A due to the association–dissociation reaction; in this case

$$Q = \tfrac{1}{3} k_d (C_3 - K C_1 C_2) \,,$$

where k_d is the dissociation constant; and K is the equilibrium constant (reciprocal weight scale).

Assuming that sedimentation begins when the components are present in an equilibrium ratio, the initial conditions may be taken to be as follows:

$$C_i(r,0) = C_i^0\Theta(r-r_0) = \begin{cases} C_i^0, & \text{for } r_0 \leqslant r < \infty \\ 0, & \text{for } r_m < r < r_0 \end{cases},$$
$$r_m < r < \infty, \quad r_m < r_0, \quad i = 1,2,3, \quad C_3^0 = KC_1^0C_2^0. \tag{5}$$

The boundary conditions are the following:

$$\begin{cases} C_i(r_m, t) = 0, \\ \dfrac{\partial C_i}{\partial r}(r_m, t) = 0, \quad i = 1,2,3, \quad 0 < t < \infty. \end{cases} \tag{6}$$

The initial conditions (5) imply that the transport process is considered after the concentration of the substance at the meniscus and its derivative becomes equal to zero. The validity of the boundary conditions (6) for velocity sedimentation is indicated by the fact that $\partial C_i/\partial t \leqslant 0$ while $C_i(r_m, 0)$ and $\partial C_i/\partial r(r_m, 0)$ are zero.

Further, let us introduce the point $\tilde{r}_i$, at the boundary of the ith component:

$$\tilde{r}_i = \sqrt{r_p^2 - (2/C_i^p)\int_{r_m}^{r_p} C_i r\,dr}, \tag{7}$$

where r_p is a certain point in the plateau region where $\partial C/\partial r$ and $\partial^2 C/\partial r^2$ are zero, and C_i^p is the concentration of the ith component in the plateau region.

Now let us determine the sedimentation coefficient of the ith component from the equation

$$\tilde{S}_i = \frac{1}{\omega^2}\frac{1}{\tilde{r}_i}\frac{d\tilde{r}_i}{dt}. \tag{8}$$

It follows from (7) and (8) that

$$\tilde{S}_i = \frac{1}{\omega^2}\frac{\dfrac{1}{C_i^p}\dfrac{dC_i^p}{dt}\displaystyle\int_{r_m}^{r_p} C_i r\,dr - \int_{r_m}^{r_p} r\frac{\partial C_i}{\partial t}\,dr}{r_p^2 C_i^p - 2\displaystyle\int_{r_m}^{r_p} C_i r\,dr} \tag{9}$$

We obtain from eq. (4)

$$\begin{aligned} \frac{dC_1^p}{dt} &= -2S_1\omega^2 C_1^p + Q^p, \\ \frac{dC_2^p}{dt} &= -2S_2\omega^2 C_2^p + 2Q^p, \\ \frac{dC_3^p}{dt} &= -2S_3\omega^2 C_3^p - 3Q^p. \end{aligned} \tag{10}$$

To compute the integral $\int_{r_m}^{r_p} r(\partial C_i/\partial t)\,dr$ in formula (9), we multiply the transport equations (4) by r and integrate them within the limit indicated, taking into account the boundary conditions (6). We then obtain

$$\int_{r_m}^{r_p} r\frac{\partial C_1}{\partial t}\,dr = -S_1\omega^2 r_p^2 C_1^p + \int_{r_m}^{r_p} Qr\,dr,$$

$$\int_{r_m}^{r_p} r\frac{\partial C_2}{\partial t}\,dr = -S_2\omega^2 r_p^2 C_2^p + 2\int_{r_m}^{r_p} Qr\,dr, \tag{11}$$

$$\int_{r_m}^{r_p} r\frac{\partial C_3}{\partial t}\,dr = -S_3\omega^2 r_p^2 C_3^p - 3\int_{r_m}^{r_p} Qr\,dr.$$

Substituting the values of (10) and (11) into (9), we have

$$\tilde{S}_1 = S_1 + \frac{1}{\omega^2}\,\frac{\frac{1}{2}Q^p(r_p^2-\tilde{r}_1^2) - \int_{r_m}^{r_p} Qr\,dr}{\tilde{r}_1^2 C_1^p},$$

$$\tilde{S}_2 = S_2 + \frac{2}{\omega^2}\,\frac{\frac{1}{2}Q^p(r_p^2-\tilde{r}_2^2) - \int_{r_m}^{r_p} Qr\,dr}{\tilde{r}_2^2 C_2^p}, \tag{12}$$

$$\tilde{S}_3 = S_3 - \frac{3}{\omega^2}\,\frac{\frac{1}{2}Q^p(r_p^2-\tilde{r}_3^2) - \int_{r_m}^{r_p} Qr\,dr}{\tilde{r}_3^2 C_3^p}.$$

Next, to determine the separation parameter, e.g., for the 3rd and 2nd components, consider the expression

$$(\tilde{S}_3-\tilde{S}_2) = (S_3-S_2) - \frac{1}{\omega^2}\left(3\,\frac{\frac{1}{2}Q^p(r_p^2-\tilde{r}_3^2) - \int_{r_m}^{r_p} Qr\,dr}{\tilde{r}_3^2 C_3^p} + 2\,\frac{\frac{1}{2}Q^p(r_p^2-\tilde{r}_2^2) - \int_{r_m}^{r_p} Qr\,dr}{\tilde{r}_2^2 C_2^p}\right). \tag{13}$$

Substituting the value of Q [see eq. (4)] into (13) and introducing the point $\tilde{r}_{1,2}$, according to the expression

$$\tilde{r}_{1,2}^2 = r_p^2 - \frac{2}{C_1^p C_2^p}\int_{r_m}^{r_p} C_1 C_2 r\,dr, \tag{14}$$

we obtain

$$(\tilde{S}_3-\tilde{S}_2) = (S_3-S_2) - \frac{k_d}{\omega^2}\left[KC_1^pC_2^p\,\frac{(\tilde{r}_3^2-\tilde{r}_{1,2}^2)}{2}\left(\frac{1}{\tilde{r}_3^2C_3^p}+\frac{2}{3}\,\frac{1}{\tilde{r}_2^2C_2^p}\right)+\frac{2}{3}\,\frac{1}{\tilde{r}_2^2C_2^p}(C_3^p-KC_1^pC_2^p)\,\frac{(\tilde{r}_3^2-\tilde{r}_2^2)}{2}\right]. \tag{15}$$

The parameter of components separation will be according to expression (2)

$$Z_{32} = \omega^2 T(S_3-S_2) - k_d\int_0^T\left[KC_1^pC_2^p\,\frac{(\tilde{r}_3^2-\tilde{r}_{1,2}^2)}{2}\left(\frac{1}{\tilde{r}_3^2C_3^p}+\frac{2}{3}\,\frac{1}{\tilde{r}_2^2C_2^p}\right)\right.$$

$$\left.+\frac{2}{3}\,\frac{1}{\tilde{r}_2^2C_2^p}\,(C_3^p-KC_1^pC_2^p)\,\frac{(\tilde{r}_3^2-\tilde{r}_2^2)}{2}\right]dt. \tag{16}$$

Let us now evaluate the effect of association–dissociation reactions on the separation of components, assuming the following approximate equalities

$$C_i^{\mathrm{p}} = C_i^0 \exp(-2S_i\omega^2 t)\,, \qquad \tilde{r}_i = r_{\mathrm{m}} \exp(S_i\omega^2 t)\,, \qquad \tilde{r}_{1,2} = \tilde{r}_2\,. \tag{17}$$

These approximations will evidently be valid if the effect of the reactions is relatively small and the components are separated. Then, after performing integration in expression (16), we have

$$Z_{32} = \omega^2 T(S_3 - S_2)\,[1 - (\tfrac{1}{2}k_{\mathrm{d}}T + \tfrac{1}{3}k_{\mathrm{a}}C_1^0 T)]\,. \tag{18}$$

Similarly, we can obtain

$$Z_{21} = \omega^2 T(S_2 - S_1)\,[1 - (\tfrac{1}{3}k_{\mathrm{d}}C_1^0 T + \tfrac{1}{6}k_{\mathrm{a}}C_2^0 T)]\,. \tag{18'}$$

It follows from (18) and (18′) that the presence of an association–dissociation reaction hinders the separation of components, the effect of this reaction being strongly dependent on the reaction rate and ultracentrifugation speed. In order that the components might be distinctly separated, it is necessary and sufficient that the following inequalities be fulfilled:

$$k_{\mathrm{d}}T \ll 1\,, \quad k_{\mathrm{a}}C_1^0 T \ll 1\,, \quad k_{\mathrm{a}}C_2^0 T \ll 1\,. \tag{19}$$

Appendix 2

2.A. Sedimentation coefficient of the reversibly interacting system $AB \rightleftarrows A + B$ as determined taking into account the kinetics of the association–dissociation reaction

The differential equations of the transport of the substance in velocity sedimentation of the reversibly interacting system $AB \rightleftarrows A + B$, as well as the initial and boundary conditions have already been presented in appendix 1 [eqs. (4)–(6)]. Let us suppose that there exists a solution to eqs. (4)–(6) and that an arbitrary point is introduced at the boundary of the concentration curve;

$$\tilde{r} = \sqrt{r_{\mathrm{p}}^2 - (2/C_{\mathrm{p}})\int_{r_{\mathrm{m}}}^{r_{\mathrm{p}}} Cr\,\mathrm{d}r}\,, \tag{20}$$

where r_{p} is a certain point in the plateau region where $\partial C/\partial r$ and $\partial^2 C/\partial r^2$ are zero, and C_{p} is the concentration of the substance in the plateau region. It can be readily shown that the point $\tilde{r}$ corresponds to the mean square second moment of the concentration gradient curve [7]. Now the sedimentation coefficient of the system can be determined in accordance with the equation

$$\tilde{S} = \frac{1}{\omega^2}\frac{1}{\tilde{r}}\frac{\mathrm{d}\tilde{r}}{\mathrm{d}t}. \tag{21}$$

Using expression (20), we obtain

$$\tilde{S} = \frac{1}{\omega^2}\,\frac{\dfrac{1}{C_{\mathrm{p}}}\dfrac{\mathrm{d}C_{\mathrm{p}}}{\mathrm{d}t}\displaystyle\int_{r_{\mathrm{m}}}^{r_{\mathrm{p}}} Cr\,\mathrm{d}r - \int_{r_{\mathrm{m}}}^{r_{\mathrm{p}}} r\frac{\partial C}{\partial t}\,\mathrm{d}r}{r_{\mathrm{p}}^2 C - 2\displaystyle\int_{r_{\mathrm{m}}}^{r_{\mathrm{p}}} Cr\,\mathrm{d}r}\,. \tag{22}$$

To calculate the integral $\int_{r_m}^{r_p} r(\partial C/\partial t)\,dr$, let us multiply the transport equations (4) by r, sum them up and integrate within the limits indicated, taking into account the boundary conditions. We shall then have

$$\int_{r_m}^{r_p} r\frac{\partial C}{\partial t}\,dr = -\omega^2 r_p^2 \sum_{i=1}^{3} S_i C_i^p \,. \tag{23}$$

On the other hand, from the transport equations (4) it can be easily obtained that

$$\frac{dC_p}{dt} = -2\omega^2 \sum_{i=1}^{3} S_i C_i^p \,. \tag{24}$$

Substituting the values of (23) and (24) into (22) we find that

$$\tilde{S} = \frac{\sum S_i C_i^p}{\sum C_i^p} \,.$$

Evidently, $\tilde{S}$ is time-dependent. For an experimental determination of the sedimentation coefficient, it is common practice to use the method of least squares which average the above relations. As a result, we obtain the sedimentation coefficient

$$\bar{S} = \frac{3}{T^3}\int_0^T t\,dt \int_0^t \tilde{S}(\tau)\,d\tau \,, \tag{26}$$

where T is the final time of sedimentation. Thus, averaging expression (25) by the method of least squares we have in first approximation

$$\bar{S} = \tilde{S}_0 - \tfrac{3}{4}\omega^2 T(\widetilde{S_0^2} - \tilde{S}_0^2) \,, \tag{27}$$

where

$$\tilde{S}_0 = \frac{\sum S_i C_i^0}{\sum C_i^0} \,, \quad \widetilde{S_0^2} = \frac{\sum S_i^2 C_i^0}{\sum C_i^0} \,.$$

It is seen from expression (27) that the sedimentation coefficient of the reversibly interacting system is equal, with good approximation, to the weight average sedimentation coefficient corresponding to the initial distribution of components and is not dependent on the rotor speed provided that $\omega^2 T = \text{const}$.

2.B. Sedimentation coefficient of the equilibrium reversibly interacting system $AB \rightleftarrows A + B$

If a reversibly interacting system remains in equilibrium at all times during sedimentation, then the following ratio of concentrations should be fulfilled:

$$C_3 = KC_1C_2 \,. \tag{28}$$

In this case, the rate Q of concentration change [see eq. (4)] of a component A as a result of the association–dissociation reaction will now be determined by the rate of concentration changes of the components due to sedimentation only. Indeed, it can be really shown from eq. (4) and the ratio (28) that

$$Q = \frac{1}{KC_2 + 2KC_1 + 3}\left.\frac{\partial C_3}{\partial t}\right|_S - \frac{KC_1}{KC_2 + 2KC_1 + 3}\left.\frac{\partial C_2}{\partial t}\right|_S - \frac{KC_2}{KC_2 + 2KC_1 + 3}\left.\frac{\partial C_1}{\partial t}\right|_S \,, \tag{29}$$

where

$$\left.\frac{\partial C_i}{\partial t}\right|_S = D_i\frac{\partial^2 C_i}{\partial r^2} + D_i\frac{1}{r}\frac{\partial C_i}{\partial r} - S_i\omega^2 r\frac{\partial C_i}{\partial r} - 2S_i\omega^2 C_i\,,$$

is the rate of concentration change of the ith component due to sedimentation. Thus, the equations of substance transport in sedimentation velocity studies of the equilibrium interacting system $AB \rightleftarrows A + B$, as well as the initial and boundary conditions will remain the same as before, and only the value of Q will change.

Performing next the operations described earlier in this appendix, we obtain

$$\tilde{S} = \frac{\sum S_i C_i^p}{\sum C_i^p}. \tag{30}$$

Averaging $\tilde{S}$ by the method of least squares will give

$$\bar{S} = \tilde{S}_0 - \tfrac{3}{4}\omega^2 T(\widetilde{S_0^2} - \tilde{S}_0^2) - \tfrac{3}{4}\omega^2 T\frac{(3S_3 - 2S_2 - S_1)(S_1 + S_2 - S_3)}{(KC_2^0 + 2KC_1^0 + 3)}\frac{C_3^0}{\sum C_i^0}. \tag{31}$$

Thus, the sedimentation coefficient of an equilibrium reversibly interacting system determined by the mean square second moment is likewise rotor speed independent and is equal, with good approximation, to the weight average sedimentation coefficient of that system with the concentration ratio of components corresponding to the equilibrium existing before the onset of sedimentation.

Appendix 3

Sedimentation coefficient of the equilibrium reversibly interacting system $AB \rightleftarrows A + B$ with a pressure-dependent equilibrium constant

The differential equations of the process will be the same as in the preceding case of velocity sedimentation of the equilibrium reversibly interacting system (appendix 2.B). However, the equilibrium constant which is pressure-dependent, will be now a function of the distance from the axis of rotation:

$$K = K_0 \exp\left[-(\Delta V/RT)\rho\omega^2(r^2 - r_m^2)\right], \tag{32}$$

where

$$\Delta V = V_{50S-30S} - (V_{50S} + V_{30S}) > 0\,, \tag{32}$$

is the change in molar volume upon association, and $V_{50S-30S}$, V_{50S}, V_{30S} are the molar volumes of the 50S–30S associate and 50S and 30S subparticles, respectively.

The initial conditions will of course change:

$$\begin{aligned} C_1(r,0) &= (3/4K)(\sqrt{1+\tfrac{8}{9}KC_0} - 1)\,\Theta(r - r_0)\,,\\ C_2(r,0) &= (3/2K)(\sqrt{1+\tfrac{8}{9}KC_0} - 1)\,\Theta(r - r_0)\,,\\ C_3(r,0) &= [C_0 - (9/4K)(\sqrt{1+\tfrac{8}{9}KC_0} - 1)]\,\Theta(r - r_0)\,, \end{aligned} \tag{33}$$

where C_0 is the initial concentration of the preparation

$$C_0 = C_1(r,0) + C_2(r,0) + C_3(r,0) \equiv \text{const}\,.$$

It is assumed here that during the time of rotor acceleration and detachment of the substance from the meniscus, the ratio of components along the cell changes in accordance with the pressure dependence of the equilibrium constant. The concentration changes of components during that time due to sedimentation are neglected because of their uncertainty and smallness. In deriving eq. (33), the initial ratio of components A and B was taken to be equimolar.

The boundary conditions are the same as before:

$$\begin{cases} C_i(r_m, t) = 0\,, \\ \dfrac{\partial C_i}{\partial r}(r_m, t) = 0\,, \quad i = 1,2,3\,, \quad 0 < t < \infty\,. \end{cases} \tag{34}$$

Proceeding from eqs. (4), (33) and (34) and taking into account the pressure dependence of the equilibrium constant (32), it is not difficult to show that, generally speaking, there can be no plateau region after the boundary. However, no bends in the plateau region are observed in sedimentation studies of the ribosomal preparations tested (see fig. 1). Also, it can be shown by numerical methods, using eqs. (4), (33) and (34), that when $\Delta V = 500 - 2000$ ml/mole, the bends of the concentration curve in the plateau region during the usual time of ultracentrifugation at high speeds (40000 r.p.m.) lie within the limits of experimental errors. Therefore as in the preceding cases, a conventional point may be introduced at the boundary according to the equation

$$\tilde{r} = r_p^2 - \frac{2}{\sum C_i^p} \int_{r_m}^{r_p} r \sum C_i \, dr\,. \tag{35}$$

However, the fixed point r_p does not, strictly speaking, belong to the plateau region in this case.

Then, let us introduce, as usual, the sedimentation coefficient

$$\tilde{S} = \frac{1}{\omega^2} \frac{1}{\tilde{r}} \frac{d\tilde{r}}{dt}\,. \tag{36}$$

Substituting now (35) into (36) we have

$$\tilde{S} = \frac{1}{\omega^2} \frac{\dfrac{1}{\sum C_i^p} \dfrac{d}{dt} \sum C_i^p \displaystyle\int_{r_m}^{r_p} r \sum C_i \, dr - \int_{r_m}^{r_p} r \frac{\partial}{\partial t} \sum C_i \, dr}{r_p^2 \sum C_i^p - 2 \displaystyle\int_{r_m}^{r_p} r \sum C_i \, dr}\,. \tag{37}$$

Using eq. (4),

$$\int_{r_m}^{r_p} r \frac{\partial}{\partial t} \sum C_i \, dr$$

can be determined:

$$\int_{r_m}^{r_p} r \frac{\partial}{\partial t} \sum C_i \, dr = r_p \frac{\partial}{\partial r} \sum D_i C_i^p - \omega^2 r_p^2 \sum S_i C_i^p\,. \tag{38}$$

To simplify further calculations, let us assume that

$$\frac{\partial}{\partial r}\sum D_i C_i^{\mathrm{p}} = 0 .$$

From the same transport equation (4) we find:

$$\frac{\mathrm{d}}{\mathrm{d}t}\sum C_i^{\mathrm{p}} = -2\omega^2 \sum S_i C_i^{\mathrm{p}} - r_{\mathrm{p}}\omega^2 \sum S_i \frac{\partial C_i^{\mathrm{p}}}{\partial r} . \tag{39}$$

Substituting (38) and (39) into (37) we finally obtain:

$$\tilde{S} = \frac{\sum S_i C_i^{\mathrm{p}}}{\sum C_i^{\mathrm{p}}} - \frac{r_{\mathrm{p}}}{2}\frac{\sum S_i \partial C_i^{\mathrm{p}}/\partial r}{\sum C_i^{\mathrm{p}}} \frac{(2/\sum C_i^{\mathrm{p}})\int_{r_{\mathrm{m}}}^{r_{\mathrm{p}}} r \sum C_i \,\mathrm{d}r}{r_{\mathrm{p}}^2 - (2/\sum C_i^{\mathrm{p}})\int_{r_{\mathrm{m}}}^{r_{\mathrm{p}}} r \sum C_i \,\mathrm{d}r} . \tag{40}$$

As can be seen, $\tilde{S}$ is, generally speaking, time-dependent; for the sake of simplicity, however, let us consider the effect of rotor speed on the sedimentation coefficient in zero approximation, i.e., at the initial moment of time; then:

$$\tilde{S}_0 = \frac{\sum S_i C_i^{\mathrm{p}}}{\sum C_i^{\mathrm{p}}}\bigg|_{t=0} - \frac{r_{\mathrm{p}}}{2}\frac{r_{\mathrm{p}}^2 - r_0^2}{r_0^2}\frac{\sum S_i \partial C_i^{\mathrm{p}}/\partial r}{\sum C_i^{\mathrm{p}}}\bigg|_{t=0} \tag{41}$$

Fig. 5 presents plots of $\tilde{S}_0$ as a function of rotor speed for three values of ΔV: 500, 1500 and 4000 ml/mole. For the calculations, the following parameters of the reversibly interacting system were used: $S_1 = 30S$, $S_2 = 50S$, $S_3 = 70S$, $r_0 = 6.0$ cm, $r_{\mathrm{p}} = 6.8$ cm, $t^0 = 20°C$, $\Sigma C_i^{\mathrm{p}}|_{t=0} = 100$ arbitrary concentration units and

$$K = 0.1 \exp\left[-(\Delta V/RT)\rho\omega^2(r^2 - r_{\mathrm{m}}^2)\right] .$$

The equilibrium constant K is expressed in reciprocal arbitrary units of concentration.

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